

energy landscapes. Further, *in silico* predictions were used to guide the design of post-translational modifications to rescue regulatory imbalances.

#### 2246-Pos Board B383

##### **Molecular Dynamics Studies on Phosphorylated and Unphosphorylated Cardiac Troponin**

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The Ca<sup>2+</sup>-sensitivity of cardiac muscle is modulated by a phosphorylation-dependent interaction between the N-terminal peptide of troponin I (TnI 1-30) and the N-terminal lobe of troponin C (TnC). This interaction enhances Ca<sup>2+</sup>-sensitivity and is abolished by PKA phosphorylation of Ser 22 and 23. The sequences of TnI involved are missing in X-ray diffraction structures. NMR has been used to define the structure of the missing peptides based on their binary complexes. Molecular dynamics (MD) offers a new approach that can analyze the entire troponin structure.

We have applied MD simulations on the Takeda et al. structure of the core domain of human cardiac troponin in the Ca<sup>2+</sup> saturated form. Simulations have been performed in explicit water and on an expanded model of the full crystallographic structure (385 aa). All simulations have been performed for at least 1  $\mu$ s with the AMBER GPU MD package in an isobaric-isothermal, NPT, ensemble. The first 31 residues of TnI, were modeled in as a linear chain according to the Howarth model. Simulations were run firstly of unphosphorylated troponin for 1  $\mu$ s followed by phosphorylation of the 1  $\mu$ s structure. After 1  $\mu$ s, further dephosphorylation and rephosphorylation simulations were run to check reversibility.

After 1  $\mu$ s of simulation the crystal structure of unphosphorylated troponin is retained and persistent conformations of the modelled-in peptides stabilize with 1-30 looping over TnC N-terminal domain making weak contacts in the Ser22/23 region. Upon phosphorylation only the N-terminal domain of TnC changed, notably slight helix reorientation and a reduced interaction of cTnC 1-80 with TnI (1-30) and the switch peptide (144-160). The orientation of TnC N-terminus relative to the IT arm changed and became more mobile. RMSF plots pinpointed the amino acids involved in these interactions and the effect on Ca<sup>2+</sup>-binding.

#### 2247-Pos Board B384

##### **Borrowing from the Platypus: Proline Substitution in Cardiac Troponin I**

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166 million years ago, egg-laying mammals branched off the evolutionary tree from their viviparous counterparts to give rise to the modern order Monotremata. There are only five extant monotremes, the platypus and four species of echidna; consequently the platypus represents a unique divergence in mammalian evolution. We investigated whether unique adaptations in cardiac sarcomere arising in these species confer physiological performance advantages. The heterotrimeric cardiac troponin complex (cTn) is a key regulator of contractile events, and confers calcium-sensitivity to the sarcomere. During ischemic-injury, protons accumulate in the myoplasm and acidify the sarcomere, resulting in markedly reduced calcium-sensitivity. Unlike its adult counterpart, force generation in fetal cardiac tissue is uniquely pH-insensitive. Replacement of the adult cardiac troponin I isoform (cTnI) with the fetal isoform (ssTnI) renders adult cardiac tissue relatively insensitive to acidification. Alignment and function studies have revealed that this insensitivity is derived almost exclusively from the histidine at position 132 in ssTnI. Substitution of histidine at the cognate position 164 in cTnI confers the same pH insensitivity to adult tissue. TNNI3, the gene encoding for cTnI, encodes for an alanine at position 164 in all known mammals, except the Platypus whose TNNI3 encodes for a proline. Prolines are biophysically intriguing due their helix-breaking nature; position 164 occurs in helix 4 of TnI, an important motif belonging

to the critical switch region of cTnI. MD simulations of the cTnI switch region interaction with the N-terminal domain of troponin C provide evidence of a conformational change in this region that may play a role in how the switch region interacts with troponin C. The functional and biophysical consequences of a cTnI-A164P replacement in the context of isolated myocytes by acute gene transfer as well as molecular dynamics simulations will be discussed.

#### 2248-Pos Board B385

##### **A Coarse-Grained Model to Study Calcium Activation of the Cardiac Thin Filament**

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Familial hypertrophic cardiomyopathy (FHC) is one of the most common heart disease caused by genetic mutations. Cardiac muscle contraction and relaxation involve regulation of crossbridge binding to the cardiac thin filament, which regulates actomyosin interactions through calcium-dependent alterations in the dynamics of cardiac troponin (cTn) and tropomyosin (Tm). An atomistic model of cTn complex interacting with Tm has been studied by our group. A more realistic model requires the inclusion of the dynamics of actin filament, which is almost 6 times larger than cTn and Tm in terms of atom numbers, and extensive sampling of the model becomes very resource-demanding. By using physics-based protein united-residue force field, we introduce a coarse-grained model to study the calcium activation of the thin filament resulting from cTn's allosteric regulation of Tm dynamics on actin. In the coarse-grained system, residues are represented by sidechain ellipsoids (SC) and peptide groups (p). Vectors between adjacent C $\alpha$ -C $\alpha$  and C $\alpha$ -SC are chosen as variables, and the velocity Verlet algorithm is used to derive the equations of motion. The time scale is much longer than that of all-atom molecular dynamics simulation because of the reduction of the degrees of freedom. The coarse-grained model is a good template for studying cardiac thin filament mutations that cause FHC, and reduces the cost of computational resources.

#### 2249-Pos Board B386

##### **An Explicitly Solvated Full Atomistic Model of the Cardiac Thin Filament and Application on the Calcium Binding Affinity Effects from Familial Hypertrophic Cardiomyopathy Linked Mutations**

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The former version of our cardiac thin filament model consisted of the troponin complex (cTn), two coiled-coil dimers of tropomyosin (Tm), and twenty-nine actin subunits. We now present the newest revision of the model to include both solvation and ionization. The model was developed to continue our study of genetic mutations in the cardiac thin filament proteins which are linked to familial hypertrophic cardiomyopathies. Binding of calcium to the cardiac troponin C subunit (cTnC) causes subtle conformational changes to propagate through the cTnC to the inhibitor subunit (cTnI) which then detaches from actin. Conformational changes propagate through to the cTnT subunit, which allow for the movement of Tm into the open position along actin. Myosin heads can bind to the seven open binding sites on actin, which upon hydrolysis of ATP leads to muscle contraction. Calcium disassociation allows for the reverse to occur, which results in muscle relaxation. Alterations in the calcium binding affinity can disrupt the natural processes of the heart. The inclusion of explicit TIP3 water solvation and an ionic concentration of 0.15 mol/L allows for the model to mimic the true conditions that the cardiac thin filament would feel. The move from implicit to explicit solvation allows us to get better individual local solvent to protein interactions; which are important when observing the N-lobe calcium binding pocket of the cTnC. We are able to compare *in silico* and *in vitro* experimental results to better understand the physiological effects from mutants, such as the R92L/W and F110V/I of the cTnT, on the calcium binding affinity compared to the wild type.